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Original communication

Basal epithelial formalin pigment deposition in the kidneys – A useful marker for ketoacidosis at autopsy

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ABSTRACT

Basal vacuolization of renal epithelial cells occurs in diabetic and alcoholic ketoacidosis, hypothermia and starvation. The vacuoles contain triglycerides. Following a case where formalin pigment deposition within these vacuoles led to the identification of ketoacidosis, a retrospective review of a further 31 cases with ketoacidosis, was undertaken. There were 24 diabetics and 7 alcoholics (age range 21–80 yrs; mean 50.9 yrs; M:F ratio = 2:1. The post-mortem interval was 1–12 days (mean – 4.5 days). Characteristic basally-located pigment surrounding vacuoles was found in 16 cases (51.6%) (14 diabetic ketoacidosis; 2 alcoholic ketoacidosis). Fifteen cases had no formalin pigment deposition. No relationship could be found between the intensity of staining and the postmortem interval, degree of putrefaction, or level of vitreous humour β-hydroxybutyrate. No staining was demonstrated in control cases matched for postmortem interval. Although formalin pigment deposition occurred in only 51.6% of cases with proven ketoacidosis at autopsy, it appeared to be a highly specific phenomenon. As these deposits were identifiable after recognizable cellular morphology had been lost due to autolysis and putrefaction, this artefact of fixation may be of particular use in suggesting the possibility of ketoacidosis in decomposed bodies with compromised histology.

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1. Introduction

The aetiology of basal vacuolization of epithelial cells of the proximal convoluted tubules of the kidney in the cortex and outer medulla remains unclear.[1] Cases have been reported in association with diabetic and alcoholic ketoacidosis, hypothermia and starvation,[2–12] and may be identified macroscopically at autopsy by renal cortical pallor.[13,14] Vacuoles have been shown to contain the neutral lipid, triglyceride.[3,4] As this could be the only indication of ketoacidosis at autopsy, this may be a very significant diagnostic finding. Unfortunately, cellular morphology is progressively lost after death; a process which may be exacerbated by the high glucose levels in diabetics,[15] resulting in almost complete loss of microscopic detail in badly decomposed bodies. One of the authors (JDG) has observed, however, that formalin pigment preferentially deposits in the areas of basal vacuolization. The results of the following case and study are reported to demonstrate the usefulness of this finding as a marker of basal vacuolization from ketoacidosis, particularly when tissue preservation is suboptimal.

2. Case report

A case of unexpected death is reported in an adult male who was found dead in bed at his home address. At autopsy there were moderate putrefactive changes. Toxicology did not reveal any lethal drugs or poisons. There were no injuries present. Although extensive putrefactive changes hindered accurate assessment of the histology (postmortem interval = 6 days), the pancreas showed patchy lobular atrophy and fibrosis and the liver focal severe steatosis, in keeping with alcohol related damage. In addition, deposits of birefringent formalin pigment were noted outlining subnuclear basal spaces in proximal convoluted tubular epithelial cells of the kidneys (Fig. 1). As this had been previously observed by one of the authors (JDG) in cases of ketoacidosis, biochemical analysis of vitreous humour was undertaken. This showed a markedly elevated glucose level of (51.3 mmol/L) and a raised level of

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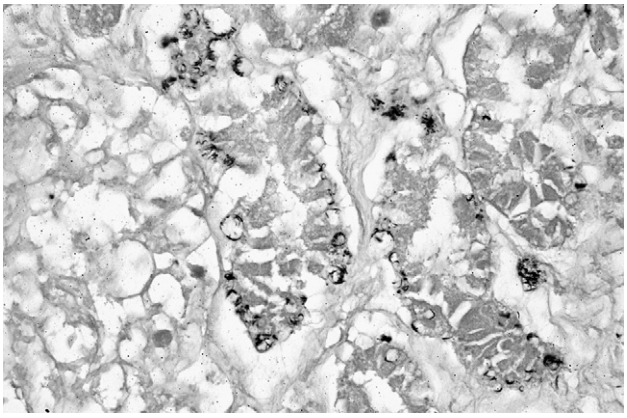

Fig. 1. Prominent formalin pigment deposition in the reported case with a prolonged postmortem interval (6 days) and marked loss of cellular detail demonstrating the usefulness of the finding in cases where cellular morphology is lacking (Haematoxylin and Eosin [H&E] × 100).

β-hydroxybutyrate (8.33 mmol/L). Death was therefore attributed to diabetic ketoacidosis. Subsequent information indicated that the deceased had been complaining of polyuria and polydipsia in the week preceding death and had a family history of diabetes mellitus.

3. Materials and methods

In view of these results, case files were retrospectively reviewed at Forensic Science SA, Adelaide, South Australia, over a six-year period from 2004 to 2009 for other cases of diabetic and alcoholic ketoacidosis. All cases had full coronial and police investigations with complete forensic autopsies. Ketoacidosis was diagnosed when the vitreous humour β-hydroxybutyrate was ≥5 mmol/L. Postmortem interval was noted. A control group matched for postmortem interval was randomly selected from case files from the same time period for comparison. All cases had renal tissue routinely sampled at autopsy and fixed in 10% buffered formalin solution for 24–48 h prior to processing for histology.

A single renal slide stained with haematoxylin and eosin from each case was evaluated blind for basal vacuolization and the presence of characteristic formalin pigment deposition: i.e. along the base of epithelial cells and outlining basal vacuoles within the proximal convoluted tubules of the kidney in the cortex and outer medulla. Cases were graded as 0 if there was no evidence of this type of basal tubular epithelial cell formalin pigment deposition, 1+ if there was mild focal deposition, 2+ if there was moderate-severe focal, or diffuse mild deposition, and 3+ if there was moderate to marked diffuse deposition. Cases were also graded from 0 to 3+ for autolytic/putrefactive changes with 0 being no autolysis or putrefaction, 1+ mild, 2+ moderate and 3+ marked, the latter with complete loss of normal cellular detail.

The amount of formalin pigment deposition was compared between the cases with ketoacidosis and the controls, and was also plotted against postmortem interval, degree of autolysis/putrefaction and β-hydroxybutyrate levels.

4. Results

A total of 31 cases were found with ketoacidosis, consisting of 24 individuals with diabetes mellitus and seven with alcoholism. The age range was 21–80 years (mean 50.9 years) with a male to female ratio of 20:11. The post-mortem interval ranged from 1 to 12 days (mean − 4.5 days). A frequent feature of basal epithelial cell formalin pigment deposition was geographic variation, with no staining in some areas of the cortex contrasting with marked staining in other areas in the same case (Fig. 2). Formalin pigment was found in 16 cases (51.6%) (in 14 cases of diabetic ketoacidosis [58.3%] and in 2 cases of alcoholic ketoacidosis [28.6%] [Fig. 3]). The number of cases without formalin pigment deposition was 15. There were 13 cases with no signs of decomposition, 7 cases with 1+ decomposition, 11 cases with 2+ decomposition. Staining was intense (≥2+) in cases with short postmortem intervals and no autolysis/putrefaction (Fig. 4), and also in cases with long postmortem intervals and marked autolysis/putrefaction, as in the reported case. No staining was demonstrated in any of the 31 control cases.

Plotting the degree of formalin pigment deposition against postmortem interval revealed no significant relationship. Similarly, no relationship could be demonstrated between the degree of autolytic/putrefactive changes and degree of formalin pigment deposition, or β-hydroxybutyrate levels and formalin pigment deposition.

5. Discussion

A variety of different types of vacuoles occur in renal tubules. Clear cell change of the proximal tubular epithelium may be caused by hyperglycemia and is known as glycogen nephrosis or Armanni Ebstein change.[16–18] Ketoacidosis from a variety of causes may result in basal vacuolization of tubular cells, with displacement of nuclei towards the lumina.[2,3] The detection of these changes on postmortem histology should raise the suspicion of an underlying metabolic disturbance and initiate biochemical analysis of vitreous humour to test for this possibility.[14] However, renal tubular epithelium quickly degrades after death due to the combined effects of autolysis and putrefaction. Thus, basal vacuolization may be obscured by cytoplasmic degeneration and lifting of cells away from the basement membrane. In the absence of a history or other indications of underlying metabolic derangements, such as alcohol abuse or diabetes mellitus, these diagnoses may go undiscovered.

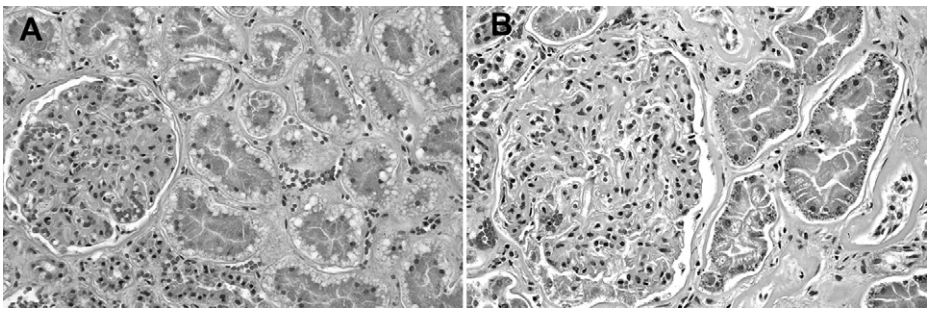

Fig. 2. Characteristic basal vacuolization of the epithelial cells of the proximal convoluted tubules of the kidney in the cortex in a case of diabetic ketoacidosis with no formalin pigment deposition (A), contrasting with adjacent areas where there was prominent deposition of pigment (B) (H&E × 100).

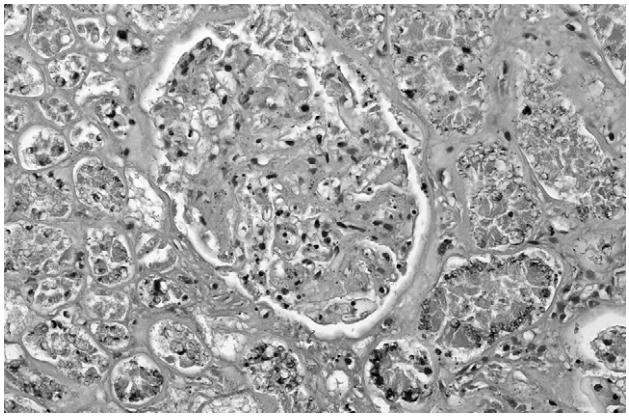

Fig. 3. Characteristic basal vacuolization of the epithelial cells of the proximal convoluted tubules of the kidney in the cortex in a case of alcoholic ketoacidosis with prominent formalin pigment deposition (H&E × 100).

Formaldehyde is used as a tissue preservative in most histology laboratories, and is commonly utilized in buffered preparations at 10% concentration. This fixative has been known to produce an artefact known as 'formalin pigment' or 'acid formaldehyde haematin', which appears as dark brown to black, finely granular, birefringent microcrystals.[19–21] These pigments are iron-free derivatives of haemoglobin, and are formed when blood-rich tissues are fixed with aqueous solutions of formaldehyde at pH less than 6.0.[20,22] Historically, these pigments have been deemed an artefact with no pathologic significance, and many techniques have been described for their prevention and removal.[19,23–25]

In 1971, Holmes reported an affinity of formalin pigment for fat, noting that it was often localized to cells which contained fat before processing of tissues in solvents. These included fatty vacuoles within cells of the liver, kidneys, and lungs.[21] The current study has demonstrated that formalin pigment also preferentially deposits in tissue sections of the kidney during fixation in areas of accumulated triglyceride. This striking aggregation of formalin pigment to the areas of triglycerides may occur because of the breakdown of triglycerides into component fatty acids, providing a localized area of reduced pH that provokes pigment deposition.

The observation that formalin pigment preferentially deposits in the vacuolated epithelial cells of the renal tubules in cases of ketoacidosis (either diabetic or alcoholic) may be significant as this may enhance the postmortem detection of metabolic disturbances. As pigment deposition may be very focal in nature, it may be useful to examine multiple areas of the cortex and outer medulla. The reason for the lack of correlation with postmortem interval and degree of decomposition is unclear, but may be related to factors that were not controlled for in this study such as minor differences in concentration of the formalin solution, amount of buffer, or time of fixation. While formalin deposition does not appear to be a particularly sensitive marker of ketoacidotic basal vacuolization (occurring in only 51.6% of cases), it does appear to be highly specific (100%). Given that these deposits are present even after autolysis and putrefaction have destroyed recognizable cellular morphology, this finding may therefore be of particular use in decomposed bodies, as was clearly demonstrated in the reported case.

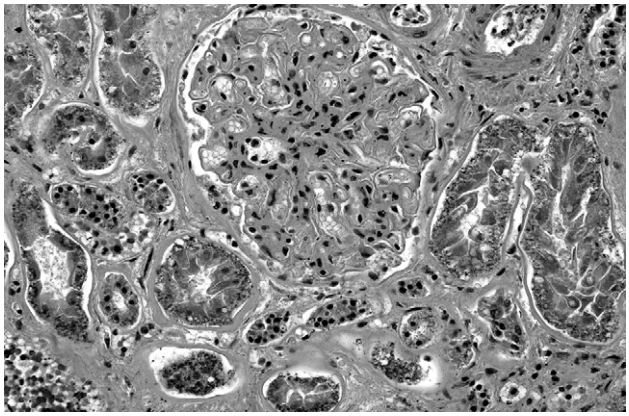

Fig. 4. Prominent formalin pigment deposition in a case with a short postmortem interval (1 day) and no autolysis or putrefaction (H&E × 100).

Ethical approval

Forensic Science South Australia.

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None.

Conflict of interest

None.

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